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ORIGINAL RESEARCH

Association Between Genetic Variations In Surfactant Protein D and Emphysema, Interstitial Pneumonia, and Lung Cancer in a Japanese Population

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Abstract

Surfactant protein D (SFTPD) is a lung-specific anti-inflammatory factor that antagonizes inflammation by inhibiting oxidative stress and stimulating innate immunity. Variations in *SFTPA2* and *SFTPB*, genes for other surfactant proteins, have been associated with lung cancer. We therefore investigated associations between *SFTPD* variations and lung cancer as well as emphysema and interstitial pneumonia, which are characterized by chronic inflammation from which lung cancer often arises. DNA from 1342 autopsy samples, including those from 140 subjects with lung cancer, was investigated. The single nucleotide polymorphism (SNP) rs721917, which results in methionine being exchanged for threonine at amino acid 11 (the Met11Thr variation), tended to be associated with emphysema and was associated with interstitial pneumonia and lung cancer. A haplotype analysis revealed that the haplotypes associated with emphysema and lung cancer differed from that associated with interstitial pneumonia, suggesting a differential role for SFTPD in the development of these diseases. A mediating analysis did not reveal a mediating effect exerted by emphysema or interstitial pneumonia on lung cancer. Our results suggested that SFTPD plays a role in the development of lung cancer and that the role for lung cancer may differ from that for interstitial pneumonia.

Keywords: Autopsy, Haplotypes, Mediation analysis, Single nucleotide polymorphism (SNP)

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Introduction

Lung cancer is a leading cause of cancer-related death worldwide (1, 2) and one of the most lethal forms of cancer in Japan (3). Genetic susceptibility in the development of lung cancer has been suggested by familial clustering and by the results of segregation analysis of lung cancer (4). Recent genome-wide association studies have suggested that variations in the genes comprising the nicotinic acetylcholine receptor gene cluster, which contains multiple genes affecting nicotine dependence of individuals, contribute to susceptibility to lung cancer in Caucasians (5–7) as well as in Japanese (8). However, whether these variations directly contribute to lung cancer or indirectly contribute to its development through smoking behavior remains controversial. Further, the genetic variations that directly affect the molecular mechanism of carcinogenesis remain largely unknown.

Chronic inflammation is a risk factor for cancer (9). The carcinogenic effect of smoking is partly mediated by the induction of diseases characterized by

chronic inflammation including emphysema (10, 11) and interstitial pneumonia (IP) (12), which frequently accompany lung cancer. A study has reported that chronic obstructive pulmonary disease (COPD) patients treated with inhaled corticosteroids have a reduced incidence of lung cancer, which suggests an important role of chronic inflammation in carcinogenesis (13). Therefore, genetic variations that affect chronic inflammation may also influence the incidence of lung cancer.

Surfactant protein D (SFTPD) is a lung-specific anti-inflammatory factor that is expressed in alveolar type II cells. It antagonizes inflammation by inhibiting oxidative stress (14) and stimulating innate immunity (15). Genetic association studies have shown that variations in the genes *SFTPA2* and *SFTPB*, which encode other surfactant proteins sharing several functions with SFTPD, are associated with lung cancer (16, 17). Furthermore, *SFTPD* variations have been associated with bronchial dysplasia, which is a known precancerous condition (18). These observations suggest that SFTPD may have a role in lung carcinogenesis.

We have previously identified an association between genetic variation in *SFTPD* and susceptibility to emphysema in Japanese patients (19). In the current study, we extended the analysis and investigated the interrelationships between *SFTPD* and emphysema, IP, and lung cancer from a genetic standpoint. For this, we focused on the single nucleotide polymorphism (SNP) rs721917 in *SFTPD*, which results in an amino acid change from Met ("T" allele) to Thr ("C" allele) at amino acid 11, and the SNP rs10887199, which does not cause any amino acid alterations but has been associated with emphysema (19, 20). We analyzed the associations of emphysema, IP, and lung cancer with these SNPs by using 1342 autopsy samples that included samples from 140 subjects with lung cancer.

Methods

Subjects

A total of 1536 patients were autopsied at the Department of Pathology, Tokyo Metropolitan Geriatric Medical Center, Tokyo, Japan, between February 1995 and July 2003. Of these, 1342 subjects who were of Japanese nationality and had a detailed pathological record of emphysema, IP, or lung cancer were enrolled in the study. The genomic DNA of each subject was used with written informed consent from a family member.

Pathological diagnosis of subjects

Each subject underwent pathological examination, and the emphysema type (centriacinar, panacinar, or paraseptal) and severity (0, none; 1, minimal; 2, mild; 3, moderate; or 4, severe) (21, 22) was determined as described in our previous report (23). Briefly, both excised lungs were infused with a 10% formalin solution at a transpulmonary pressure of 25 cm H_2O. Sagittal slices approximately 2 cm thick were obtained. The lung slices were macroscopically assessed for emphysematous changes according to type and severity, and 3 pathologists (TA, TK, and MS) independently scored them without prior interpretation.

The scoring was confirmed to be devoid of inter- and intra-observer differences. The mean scores of the 3 pathologists for all slices of both lungs were used in the current study. Subjects with the centriacinar type with a severity of 3 or 4, and/or the panacinar type with a severity of >1, were included in the emphysema group. The main phenotypes of emphysema are centriacinar, panacinar, and paraseptal. We excluded the paraseptal type and only included the centriacinar and panacinar types because centriacinar emphysema is caused mainly by smoking and panacinar emphysema with centriacinar emphysema is an aggravated form of centriacinar emphysema (24). Subjects who lacked centriacinar- or panacinar-type regions were included in the non-emphysema group.

The diagnosis of IP was based on the clinical and autopsy findings of TK, MS, and TA. The IP cases included idiopathic, acute, and collagen disease-associated IPs (25, 26). Subjects with sarcoidosis and pneumoconiosis such as asbestosis were not included. We analyzed all clinical and pathological data to determine the presence or absence of lung cancer. We included cancers incidentally found at autopsy.

Genotyping

Genomic DNA was extracted from the renal cortex by using the phenol–chloroform method and stored at -20°C until use. The SNPs described above were genotyped using an ABI TaqMan® SNP genotyping assay (Life Technologies Japan, Tokyo, Japan) as previously described (27).

Statistical analysis

All values are presented as mean (SD). The differences observed between groups were compared using the unpaired *t*-test, Wilcoxon test, or Pearson's χ^2 test. Statistical analyses were performed using JMP genomics software version 3.1 (SAS Institute Inc., Cary, NC, USA) or the Statistical Package for the Social Sciences (SPSS) for Windows version 11.0.1 (SPSS Inc., Chicago, IL, USA).

The effect of each genotype of rs721917 on emphysema, IP, or lung cancer was calculated using multivariate logistic regression with a dominant, recessive, or additive model based on the "T" allele (the minor allele). The adjusted odds ratio in each model was calculated as follows: In the dominant model, the ratio of the adjusted odds for the "T/T" plus "T/C" genotypes to that for the "C/C" genotype was evaluated. In the recessive model, the ratio of the adjusted odds for the "T/T" genotype to that for the "T/C" plus "C/C" genotypes was evaluated. In the additive model, the increment of the adjusted odds ratio per one "T" allele was evaluated. The effect of

each genotype of rs10887199 was similarly evaluated on the basis of the "C" allele (the minor allele).

We estimated the unobserved haplotype frequencies by the expectation–maximization algorithm that assumes the Hardy–Weinberg equilibrium and then speculated the haplotype for each subject. The effect of each haplotype on emphysema, IP, or lung cancer was calculated using multivariate logistic regression with the additive model, in which age, gender, smoking status, and pack-years were used as covariates. The odds ratio (OR) for the development of emphysema, IP, or lung cancer according to the copy number of a chromosomal fragment with a specific haplotype was determined using a logistic regression model adjusted for age, sex, smoking status, and pack-years.

A mediation analysis was performed for the identification of a potential mediating effect that originates from the genetic variation in *SFTPD* and influences lung cancer through emphysema or IP (28, 29). In the current study, the initial variable was the rs721917 genotype, the mediator was the presence of either emphysema or IP, and the outcome was lung cancer (30, 31). First, a suitable model for the regression analysis was selected on the basis of the result of the association study, i.e., the additive model for emphysema and the recessive model for IP. In the additive model, the rs721917 genotype was coded as 0, 1, or 2 according to the number of the "T" allele. In the recessive model, the rs721917 genotype "T/T" was coded as 1 or 0. Second, the regression analysis adjusted for age, gender, smoking status, and pack-years was performed using 3 different conditions: (1) the initial variable was an independent variable and the outcome was a dependent variable; (2) the initial variable was an independent variable and the mediator was a dependent variable; and (3) both the initial variable and the mediator were independent variables and the outcome was a dependent variable. Then, the mediating effect was calculated using the β parameter estimates obtained in each condition. The significance of the mediating effect was tested using the Sobel test (30, 32). A p-value of <0.05 was considered significant.

Ethical considerations

The current study was approved by the ethics committees of Nippon Medical School (approval no. 18-11-31), Tokyo Medical and Dental University (approval no. 4), and Tokyo Metropolitan Geriatric Medical Center (approval no. 440).

Results

Subjects

The characteristics of the subjects are shown in Table 1 The presence of emphysema was significantly associated with lung cancer. The lung-cancer group had a greater number of smokers, smokers with a greater number of pack-years, and a greater proportion of males than the group without lung cancer. We thus performed regression analyses with adjustments for age, gender, and pack-years. The prevalence of IP in this autopsy population was high. One of the reasons is thought to be a bias in the study population. Tokyo Metropolitan Geriatric Medical Center is a core hospital with a ward for respiratory medicine, and the proportion of IP is expected to be higher than that in the general population. Furthermore, the morbidity of idiopathic pulmonary fibrosis (IPF) is reported to be higher in aged populations (33); therefore, the relatively high age of this population might also have contributed to the higher proportion of subjects with IP including IPF.

SNP selection

We selected 2 SNPs (rs721917 and rs10887199) from the *SFTPD* gene region that have been associated with COPD (19, 20) (Supplementary Figure 1). The SNP rs721917 (T→C) is of great interest because it causes an amino acid change (Met11Thr) that results in a reduced serum concentration of the *SFTPD* protein product as well as a lower oligomerization ability and reduced binding to bacteria of SFTPD (34, 35). The SNP rs10887199 is not located in the coding region but has been associated with COPD in our previous study (19). These SNPs have a large minor allele frequency in the Japanese population (0.477 for the "T" allele of rs721917 and 0.344 for the "C" allele of rs10887199; HapMap database [http://www.hapmap.org]) and are thus suitable for use in an association study.

Association of SFTPD SNPs with emphysema, IP, and lung cancer

The genotypes of *SFTPD* found in subjects with emphysema, IP, and lung cancer are shown in Supplementary Table 1. We used the dominant, recessive, and additive models to investigate the effect of the SNP. In the dominant model, a single copy of the risk allele exerts a full effect. In the recessive model, 2 copies of the risk allele are required to produce a full effect, while a single copy does not have any effect. In the additive model, 2 copies of the risk allele are required for a full effect, while a single copy provides half of the full effect. Then, we investigated whether any of the models elucidated the association between each SNP and the development of emphysema, IP, or lung cancer. We found that rs721917 tended to be associated with emphysema ($p = 0.066$) in the additive model, although this association was not significant (Table 2).

We also found that rs721917 was associated with IP in the recessive model ($p = 0.025$) and with lung cancer in all models ($p = 0.01$–0.005), among which the additive model had the smallest p-value (Table 2). The rs10887199 SNP did not show any association in the current study. The risk allele of rs721917 for emphysema was probably the "C" allele (Figure 1A), for IP was the "T" allele (Figure 1B), and for lung cancer was the "C" allele (Figure 1C). These results suggest that

Table 1. Genotypes of SFTPD in the subjects with IP, emphysema, and lung cancer

	Subjects with lung cancer (n = 140)	Subjects without lung cancer (n = 1202)	*p*-value
Age (mean [SD])	79.6 (7.6)	80.4 (9.0)	0.274
Gender			
Male	88 (62.9%)	627 (52.2%)	***0.016***
Female	52 (37.1%)	575 (47.8%)	
Smoking status			
Ex + current	108 (78.8%)	531 (48.0%)	***<0.0001***
None	29 (21.2%)	575 (52.0%)	
Pack-years (mean [SD])	60.1 (38.6)	48.2 (34.9)	***0.003***
Emphysema	31 (22.1%)	129 (10.7%)	***<0.0001***
IP	11 (7.9%)	82 (6.8%)	0.648
IPF/UIP	5 (3.6%)	47 (3.9%)	0.433
AIP/DAD	1 (0.7)	18 (1.5%)	0.458
Collagen Disease-associated	0	5 (0.4%)	0.445
Drug-induced	0	1 (0.1%)	0.733
Organizing pneumonia	0	17 (1.1%)	0.157
Unclassified	2 (1.4%)	12 (1.0%)	
Lung cancer			
Squamous cell carcinoma	61 (43.6%)		
Adenocarcinoma	41 (29.3%)		
Adeno-squamous cell carcinoma	7 (5.0%)		
Small-cell carcinoma	28 (20.0%)		
Large-cell carcinoma	4 (2.9%)		
Carcinoid	5 (3.6%)		

IPF, idiopathic pulmonary fibrosis; UIP, usual interstitial pneumonia; AIP, acute interstitial pneumonia; DAD, diffuse alveolar damage.

the mechanism of involvement of *SFTPD* in emphysema may be the same as that in lung cancer, while the mechanism of *SFTPD* involvement in IP differs from that in lung cancer.

Association of SNP haplotypes at the rs721917/ rs10887199 region with emphysema, IP, and lung cancer

In the analysis described here, the rs721917 "C" allele tended to be associated with emphysema and was associated with lung cancer, while the rs721917 "T" allele was associated with IP. To further confirm this result, we investigated the associations of their haplotypes with these diseases. As shown in Supplementary Figure 1, rs721917 and rs10887199 belong to the same linkage disequilibrium block and are thus suitable for determining the local haplotype. The calculation indicated that the rs721917-C/rs10887199-C haplotype is associated with emphysema, while the rs721917-T/rs10887199-C haplotype is associated with IP (Table 3). This again indicated that the rs721917 genotype associated with emphysema differed from that associated with IP. Lung cancer was associated with both "T/T" and "C/T" haplotypes (Table 3). This indicates that the rs10887199 "T" genotype may also have an effect on lung cancer rather than the rs721917 genotype.

Mediating effect analysis

In some cases, a factor exerts the final effect through an intermediate phenotype. In other words, a factor causes an intermediate phenotype, and the intermediate phenotype causes the final phenotype. The effect that a

Table 2. Associations of *SFTPD* with emphysema, IP, and lung cancer

	rs721917		rs10887199
	p-value	Adjusted odds ratio (95% CI)	p-value
Emphysema			
Dominant	0.148		0.209
Recessive	0.118		0.127
Additive	0.066		0.093
Interstitial pneumonia			
Dominant	0.873		0.666
Recessive	***0.025***	***1.98 (1.10–3.41)***	0.181
Additive	0.282		0.664
Lung cancer			
Dominant	***0.01***	***0.86 (0.34–0.91)***	0.825
Recessive	***0.044***	***0.40 (0.18–0.81)***	0.885
Additive	***0.004***	***0.62 (0.45–0.85)***	0.819

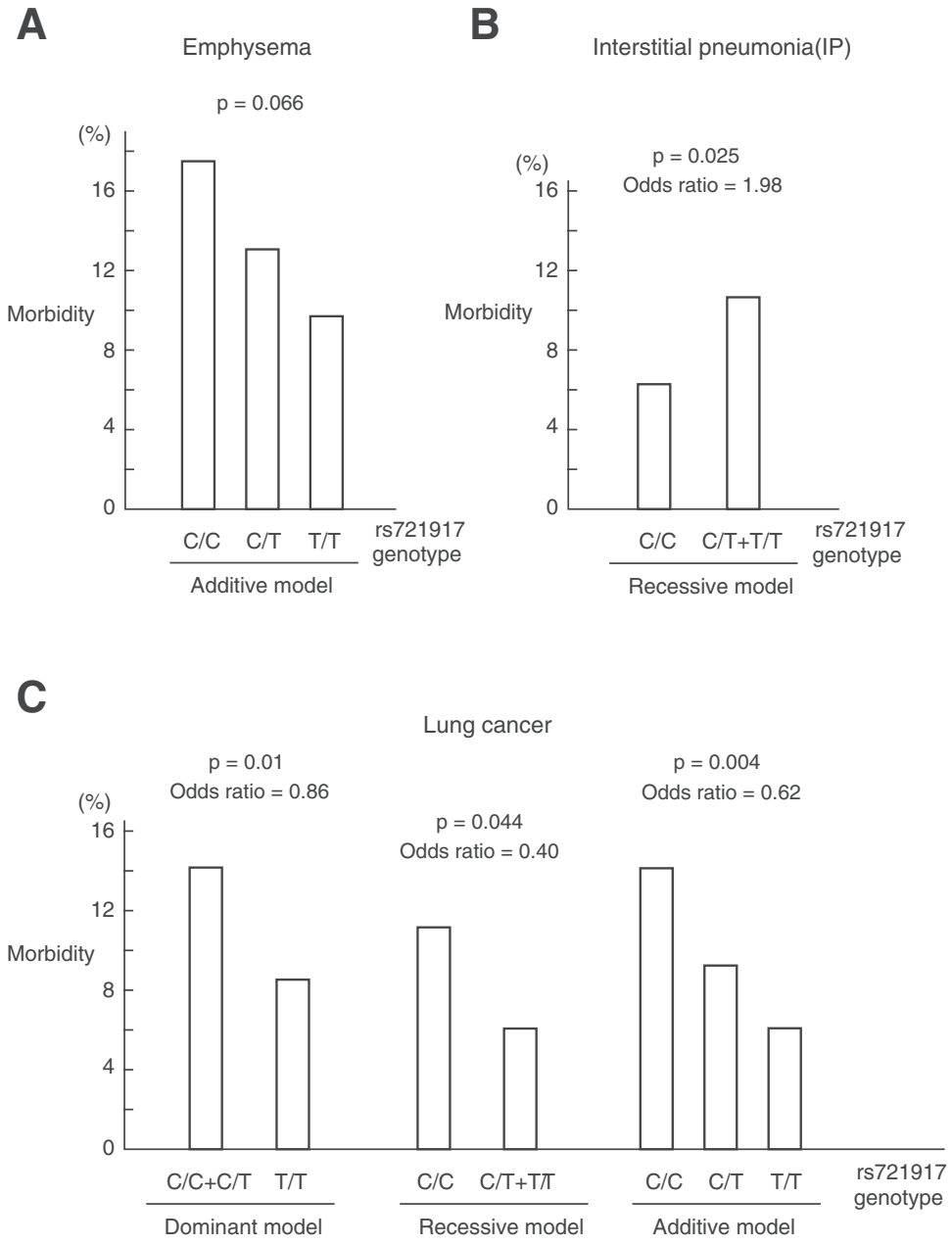

Figure 1. Association study for rs721917. **(A)** Emphysema with the additive model. **(B)** IP with the recessive model. **(C)** Lung cancer with the dominant, recessive, and additive models. Odds ratios are shown for models in which significant associations were observed.

Table 3. Associations of the haplotype at the rs721917/rs10887199 region with emphysema, interstitial pneumonia, and lung cancer

Haplotype (rs721917/rs10887199)	Haplotype frequency		IP	Emphysema	Lung cancer
		Global p-value	0.12	0.08	***0.01***
C/C	0.38	beta (SE)	–0.09 (0.36)	0.74 (0.3167)	0.18 (0.30)
		p-value	0.81	***0.02***	0.56
T/T	0.37	beta (SE)	–0.07 (0.37)	–0.29 (0.34)	–0.88 (0.34)
		p-value	0.85	0.39	***0.006***
C/T	0.22	beta (SE)	–0.17 (0.42)	–0.44 (0.38)	0.91 (0.31)
		p-value	0.68169	0.24	***0.009***
T/C	0.04	beta (SE)	1.63 (0.67)	–1.20 (0.93)	–0.92 (0.93)
		p-value	***0.02***	0.20	0.32

Significant correlations are shown in bold. SE, standard error.

factor exerts on the final phenotype via an intermediate phenotype is called a mediating effect. At rs1051730 in the CHRNA5–3 region, COPD has been reported to be a mediator of lung cancer (29). We tested our hypothesis that variations in *SFTPD* may be mediated by emphysema or IP, which are phenotypes characterized by chronic inflammation. A mediation analysis was employed to statistically test the presence of a mediating effect (Supplementary Figure 2).

We were unable to detect a statistically significant mediating effect of emphysema or IP (Table 4). This suggests that the mediating effect, if it exists at all, is not substantial.

Discussion

In the current study, we tested whether genetic variations in *SFTPD* are directly associated with lung cancer or indirectly contribute to lung cancer development through effects mediated by diseases that are characterized by chronic inflammation. The study employed 1342 consecutive autopsy subjects including 140 subjects with lung cancer. We observed a significant association of IP and lung cancer with a coding SNP that causes a Met11Thr amino acid change. The risk haplotypes of IP and lung cancer differed from that for emphysema. We were unable to detect a significant mediating effect of the *SFTPD* genotype on lung cancer through emphysema or IP.

Table 4. Mediation effect

	Estimate	SE	*p*-value
Model			
Initial factor: SNP rs721917 genotype Mediator: Emphysema Outcome: Lung cancer			
Effect			
Total effect: SNP on lung cancer	0.14	0.06	***0.006***
Effect 1: SNP on emphysema	0.09	0.05	0.07
Effect 2 (with given SNP): Emphysema on lung cancer	0.04	0.02	***0.04***
Mediation effect: SNP on lung cancer through emphysema	0.004	n.a.	0.17
Model			
Initial factor: SNP rs721917 genotype Mediator: IP Outcome: Lung cancer			
Effect			
Total effect: SNP on lung cancer	–0.64	0.32	0.04
Effect 1: SNP on IP	0.59	0.26	0.03
Effect 2 (with given SNP): IP on lung cancer	0.04	0.17	0.83
Mediation effect: SNP on lung cancer through IP	0.02	n.a.	0.83

The results of the association analyses for genotype and haplotype suggested that the role of *SFTPD* in the development of emphysema and lung cancer may differ from that in the development of IP.

Oxidative stress and bacterial infections are risk factors for emphysema (14). The "T" allele of rs721917, which increases the serum concentration of SFTPD and produces SFTPD protein with a greater oligomerization ability and a greater ability to bind to bacteria (34, 35), may prevent emphysema through an increased level of protein activity. Because the "C" allele of the rs721917 SNP was associated with both emphysema and cancer and the risk haplotypes for lung cancer differed from those for emphysema, a SNP other than rs721917, which is correlated not to "C/T" but to "C/C", might also be associated with emphysema.

Two haplotypes in the *SFTPD* gene were associated with lung cancer in our study. The circulating level of SFTPD protein has recently been reported to be associated with subsequent lung cancer risk (36), which also suggests an association between *SFTPD* and lung cancer. Although the haplotypes including the "T" allele of rs10887199 were associated with lung cancer, the SNP rs10887199 itself was not associated with this disease. One possible explanation is that these haplotypes are associated with lung cancer through different causal SNPs, which are not strongly correlated to rs10887199. Several groups suggested that different SNPs in one single gene are associated with diseases, e.g., cystic fibrosis, dyslipidemia, and lung cancer (37–39).

The carbohydrate recognition domain of SFTPD protein binds deleted in malignant brain tumors 1 (DMBT1), a tumor suppressor, and the expression of DMBT1 is frequently lost in lung cancer (40, 41). One of the possible mechanisms of the association of these *SFTPD* haplotypes and lung cancer is that these haplotypes may result in changes in the carbohydrate recognition domain, which affects the binding of SFTPD to DMBT1 and may reduce the tumor-suppressing capability of DMBT1.

Endoplasmic reticulum (ER) stress is possibly involved in the development of IP. A mutation in the SFTPC precursor protein has been reported to result in the accumulation of unfolded proteins in the ER, thereby increasing ER stress (42, 43). The rs721917-T/rs10887199-C haplotype might tag a nucleotide change that causes a structural defect in the SFTPD protein.

There are some limitations in this study. First, we were unable to demonstrate a significant mediating effect of emphysema or IP on lung cancer; this may be due to the small sample size used in the current study. A study with a larger sample size may be needed for the detection of a weak mediating effect (30). Second, we did not correct the results for multiple testing in this study. If Bonferroni corrections are performed, the thresholds of the p-value after correction for the numbers of SNPs,

diseases, and genetic models, and for the numbers of both haplotypes and genetic models, are $0.05/(2 \times 3 \times 3) = 0.002$ and $0.05/(3 \times 3) = 0.005$, respectively.

According to these strict thresholds, we cannot observe significant associations between the SNPs and the diseases in Tables 2 and 3. An analysis of the association between genetic variations of these SNPs and the diseases by two-way analysis of variance with Tukey–Kramer's honestly significant difference test and with Bonferroni's adjustment for the number of the diseases provided a significant association between rs721917 and lung cancer. Another independent population would be needed to validate our results on the association between the genetic variations of *SFTPD* and emphysema, IP, and lung cancer.

Conclusions

In conclusion, genetic variation in *SFTPD* is a risk factor for the development of IP and lung cancer. Our observation that the genetic variation associated with emphysema and lung cancer differs from that associated with IP suggests differential roles for *SFTPD* in the development of these diseases.

Declaration of Interests

The authors have no conflicts of interest to disclose. A professional editing company, Editage, reviewed the manuscript for correct English usage. The authors are responsible for the content and the writing of this paper. This study was partly supported by a grant from the Grant-in-Aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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Appendix (Online Supplemental Material):

Table S1. Genotypes of SFTPD in the subjects with IP, emphysema, and lung cancer

SNP (Genotype)	Number of subjects for each genotype			
	Emphysema	IP	Lung cancer	Whole population
rs721917 (CC/CT/TT)	66/78/17	34/40/21	64/65/13	455/709/205
rs10887199 (TT/TC/CC)	51/78/33	35/39/22	51/70/28	477/670/237